Supplemental Methods and Results 1

To intuitively visualize and extract biological meaning from these molecular profiling data, we developed the notional representation of a literal proteomic connectivity map as an alternative to heat maps and clustering. In this representation, all biological replicates are collapsed into nodes and the summaries of correlations are represented as edges, with their associated weights proportional to correlation. Only correlations above a certain threshold are plotted for visual clarity. A spring-embedded, force-directed model (with spring strength proportional to edge weight) is used to organize the map. Thus, perturbations with strong similarity at the molecular profile level will be proximal while those with weaker correlation will be distal. Color coding of the nodes by compounds class and cell type provides an additional layer of visual information.

We find that this network visualization provides an easy way to grasp large proteomic data sets and such maps can lead to new functional insights or hypotheses. Regions of the map derived from the data shown in **Fig. 4A** are rendered in **Supp. Figs. 2A** and **2B**. For example, the map intuitively demonstrates correlations among different compound perturbations within *and across* cell types. A subnetwork containing all of the cardiac glycoside treatments of MCF7 and PC3 is shown to have tight connectivity, as expected, in **Supp. Fig. 2A**. Through this analysis, we discovered the possible connection of the protein synthesis inhibitor, anisomycin, and the CDK2 inhibitor/insulin production agonist, GW8510, to the cardiac glycosides. This novel insight might suggest further uses or side effects of these compounds.

The connectivity of several cardiac glycosides within the HL60 cell type is shown in **Supp. Fig. 2B**. Interestingly, the chemotherapeutic agents doxorubicin and daunorubicin (drugs with cardiotoxicity that was identified after they entered therapeutic practice) also connect to the cardiac glycosides, perhaps suggesting a possible mechanism of their deleterious side effects[42,43]. By comparison of the distances in **Supp. Figs. 2A** to **2B**, we can also intuit that these

connections are stronger in the leukemic cell type (HL60) than they are in the solid tumor cells (MCF7, PC3). A further interesting point is that P100 molecular signatures connect the DNA intercalation agents (doxorubicin and daunorubicin) with a DNA crosslinking agent (carmustine), two molecular mechanisms of action that are likely to have some cellular effects in common[44,45].

Supplemental Figure 2 Legend:

Supplemental Fig. 2: Connectivity Map visualizations of P100 confirmatory data. A "Connectivity Map" of drug perturbations within 3 cell lines created using the correlations of P100 measurements is depicted in E and F. Each node is colored internally by the class of treatment and externally by the cell type. The distances between nodes are calculated based on P100 molecular signature Pearson correlation coefficients and degree of connectivity. In A), a subnetwork that contains all of the cardiac glycoside treatments of MCF7 and PC3 cells is shown to have tight connectivity. B) depicts the connectivity of several cardiac glycosides in HL60 cells.